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Supporting Information

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The Tn Antigen—Structural Simplicity and Biological Complexity

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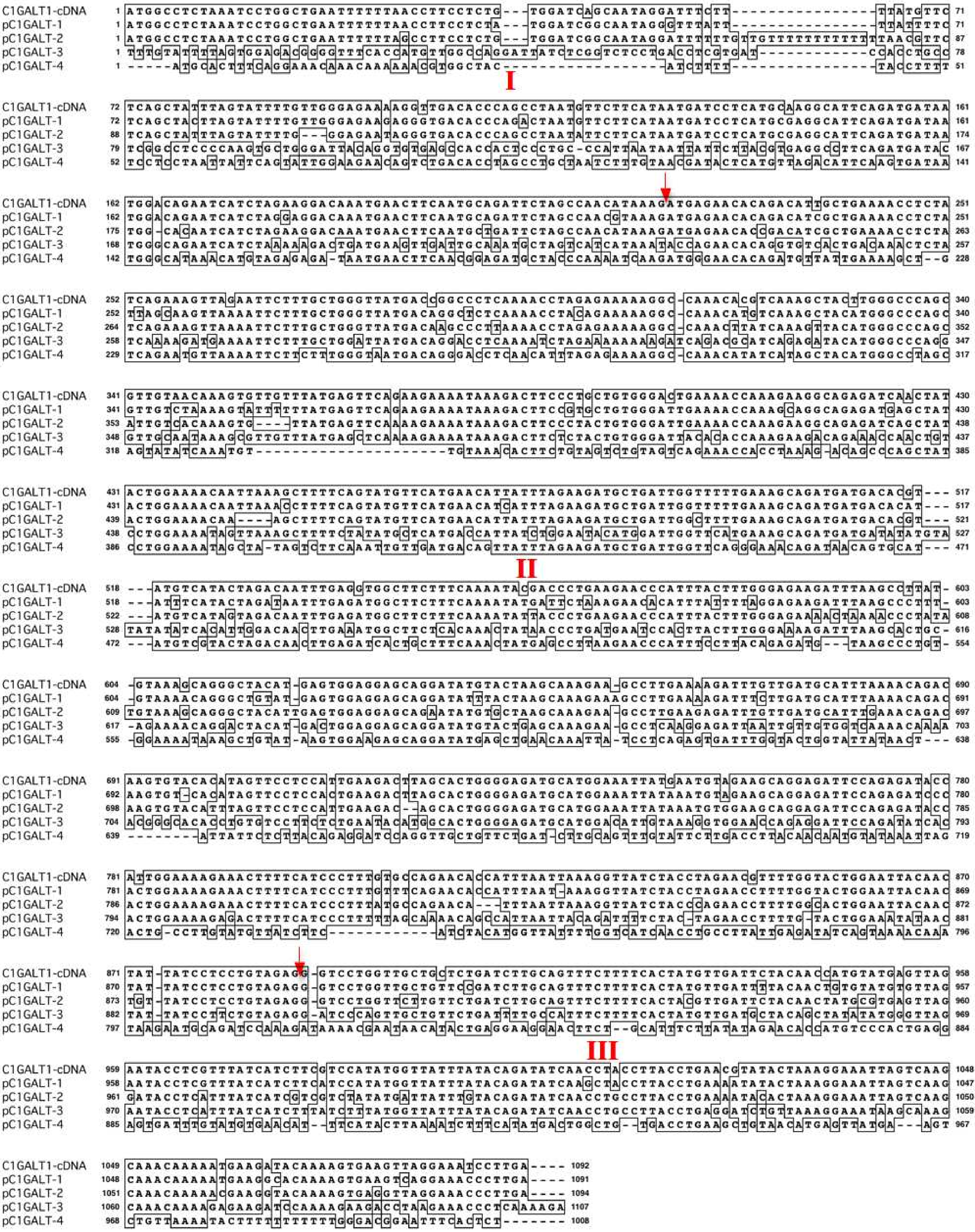

C1GALT1-cDNA
pC1GALT-1
pC1GALT-2
pC1GALT-3
pC1GALT-4
I
II
III

Supplemental Figure 4. Alignment of human *T-synthase* pseudogenes to the cDNA of the functional gene. The homologous region of the human pseudogenes, *pC1GALT-1, -2, -3*, and *-4* were aligned to the open reading frame of the cDNA of the functional T-synthase (*C1GALT1*) using the MacVector9.5 DNA analyzing program. The numbering of nucleotides is shown on both sides of the sequence. The boundaries of the three exons (Exon I, II, and III) are indicated by arrows. Identical sequences are boxed.

Supporting information: Protein Recognition by an Ensemble of Fluorescent DNA G-Quadruplexes

*David Margulies and Andrew D. Hamilton**

1. General. ^{1}H NMR spectra were acquired on Bruker Advance DRX 400 series spectrophotometers at 400 MHz. Fluorescence measurements were carried out using Hitachi F-4500 spectrometer with excitation slit 10 mm. Formation of quadruplexes was verified by circular dichroism spectrophotometry using an Aviv62DS spectrapolarimeter with a 1 mm pathlength cuvette. In all cases data was subtracted from the spectra of a solution containing only buffer or water. A single drop fluorescence measurement was performed using NanoDrop 3300 fluorospectrometer. Reagents, salts, and proteins were purchased from Sigma-Aldrich, except lipase that was purchased from MP biomedical. Proteins were dissolved in buffer or water; desalted using Bio spin 30 tris columns and their concentrations were determined using their molecular weights and molar absorption coefficients at 276-280 nm. For mouse-MBP and unfractionated whole histone, average values were taken. Oligonucleotides modified with fluorescein, tamra and *N*-hydroxysuccinimide ester were synthesized by the W. M. Keck Foundation Biotechnology Resource Laboratory located at Yale University, using standard automated solid phase synthesis. Concentration of the oligonucleotide was obtained from UV measurements at 260 nm using an Agilent A453 spectropolarimeter, based on their respective electronic absorption at 260 nm and their molar extinction coefficients obtained by nearest neighbor calculations. Extinction coefficients of the dyes were taken as 38,800 $M^{-1}cm^{-1}$ fluorescein-dT, 32,300 $M^{-1}cm^{-1}$ tamra (Glenresearch), and 22300 $M^{-1}cm^{-1}$ for pyrene. Oligonucleotides were characterized by Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) spectrometry (Applied Biosystems Voyager –DE PRO Workstation). Chromatographic separation was achieved by reverse-phase HPLC on a Varian Prostar equipped with a Timberline TL-105 column heater.

2. Preparation of oligonucleotides. Synthesis of amino modified pyrene: 1-pyrene carboxylic acid (100 mg, 0.4 mmol) was dissolved in oxalyl chloride (0.7 mL, 8.12 mmol) and DCM (4 mL). Two drops of DMF were added and the solution was stirred under nitrogen overnight. The solvent was evaporated, DIPEA (0.1 mL, 0.6 mmol) and N-Boc-1,5-diaminopentane (0.43 mL, 2 mmol) in DCM were added and the solution was stirred for 18 hours. The solvent was removed; the residue was dissolved in DCM, washed several times with 1N $NaHCO_3$ and 0.5N HCl, dried and evaporated. The crude product was purified by flash chromatography using 2.5% methanol in DCM as eluents. It was dissolved in DCM (5 mL) followed by addition of TFA (5 mL) and 2 drops of water to the solution. After a few hours the solvent was removed. TFA was co-evaporated with DCM and methanol for several times, followed by evaporation of the solvent under high vacuum to afford the amino modified pyrene in 63% yield. 1H NMR (400 MHz, CD_3OD) δ = 1.58 (m, 2H, $C\underline{H_2}CH_2CH_2$), 1.78 (m, 4H, $CH_2C\underline{H_2}N$, $NC\underline{H_2}CH_2$), 2.98 (t, J=7.6 Hz, 2H, $C\underline{H_2}NH_2$), 3.57 (t, J=7.2 Hz, 2H, $C\underline{H_2}NHCO$), 8.06-8.27 (m, 8H, Pyr), 8.43 (d, J=8.0 Hz, 1H, Pyr). *MS-ES+:(m/z)* 331.05.

Synthesis of pyrene appended ODN: to a vial containing 6 mg of amine 1 ml of anhydrous DMF, and 100 µL anhydrous DIPEA were added. The resulting solution was transferred into a syringe and introduced into a cartridge containing a solid support coupled to *N*-hydroxysuccinimide ester (NHS ester) modified oligonucleotide (1.0 µmol scale). The amine solution was pushed through the cartridge several times and then the resin-linked DNA/amine mixture was agitated overnight. The amine solution was then removed and the cartridge was washed three times with 1mL DMF followed by 1mL aliquots of HPLC grade acetonitrile. The resin linked DNA was dried by introducing argon flow through the cartridge for 1 hr. Cleavage from the resin and global deprotection was achieved by introducing a 30% solution of NH_4OH (3 ml) and incubating the solution overnight at 55C. The solvent was evaporated and the solution was purified by gel filtration using Microspin G-25 Columns (Amersham Biosciences). Further purification of pyrene appended oligonucleotides was achieved using a Varian PRP-1 reverse-phase HPLC column that was maintained at 65°C using a heat jacket. The sample was heated to 95C for 10 min, and then rapidly introduced into the HPLC injector. The solvent system used was A: 0.1M TEAA, 5% ACN, B: 100% ACN. The pyrene modified ODN was checked by MALDI-TOF. Calculated monoanion; 2864.07, observed: 2862.4. Commercially available fluorescein and tamra appended ODNs were dissolved in water and further purified by gel filtration using Microspin G-25 Columns (Amersham Biosciences). Calculated fluorescein monoanion; 3008.15, observed: 3007.2. Calculated tamra monoanion: 3063.29, observed: 3062.3.

3. Formation of quadruplex ensembles and protein detection. Stock solutions of PFT quadruplex ensemble were prepared by mixing the three ODNs in a minimum amount of water (<10µL), followed by addition of 130 µL, of 10 mM Tris-HCl, 80 mM KCl, pH 7.3, such that the final concentrations of ODNs resulted in 40 µM P and 44 µM F and 44 µM T (32 µM quadruplexes). After fastening the cap and sealing with parafilm quadruplex formation was initiated by heating the solution at 97C for 15min, followed by slow cooling to room temperature and 48h incubation at 4C. The PFT* ensemble was prepared by heating a water solution, containing the same ODNs concentrations, to 97C for 15, followed by slow cooling to room temperature. The solution was then heated again for 15 minutes, removed from the heater and rapidly evaporated to dryness under vacuum. The solid was dissolved in 10mM Tris-HCl, 80mM KCl, pH 7.3, and quadruplex formation was achieved by following by the same protocol as for PFT. Before each fluorescence titration, fresh quadruplex stock solutions were diluted by 70 folds with buffer. In a single microliter measurement, a drop of buffer is first measured as blank, after which drops of PFT or PFT loaded with a protein are measured.

4. Principal component analysis. Analysis of the emission spectra was performed by XLSTAT 7.5.3 - Principal Component Analysis (PCA) on 57 data points corresponding to the change in emission $[(I_{protein}-I_{ref}) / I_{ref}]*100$ at 370-650 nm every 5 nm.